

WHAT IS CLAIMED IS:

1. A method of regulating expression of a gene in a prokaryotic cell, the method comprising:

5 providing a prokaryotic cell comprising a nucleic acid encoding an artificial polypeptide, wherein the artificial polypeptide comprises a zinc finger domain, and wherein the artificial polypeptide binds to a target DNA site in a gene;

expressing the nucleic acid encoding the artificial polypeptide in the cell under conditions in which the artificial polypeptide is produced, binds to the target DNA site, and regulates the gene..

2. The method of claim 1, wherein the artificial polypeptide comprises at least three zinc finger domains.

3. The method of claim 1, wherein the gene is an endogenous gene.

4. The method of claim 3, wherein expression of two or more endogenous genes is regulated.

5. The method of claim 4, wherein the artificial polypeptide regulates expression of a polycistronic RNA.

6. The method of claim 1, wherein expression of the gene is repressed relative to expression of the gene in the absence of the artificial protein.

7. The method of claim 1, wherein the cell is an *E. coli* cell.

8. The method of claim 1, wherein the regulating alters a trait of the cell relative to a reference cell.

9. The method of claim 8, wherein the trait is heat resistance or solvent resistance.

10. The method of claim 3, wherein the endogenous gene encodes a decarboxylase enzyme.

11. The method of claim 10, wherein the decarboxylase enzyme is a
5 decarboxylase enzyme of a ubiquinone biosynthetic pathway.

12. The method of claim 11, wherein the enzyme is a ubiX gene product.

13. The method of claim 1, wherein expression of the nucleic acid encoding the
10 artificial polypeptide is regulatable.

14. The method of claim 3, further comprising characterizing the endogenous gene.

15. The method of claim 14, wherein the characterizing comprises identifying
15 DNA bound by the artificial polypeptide, and determining the nucleotide sequence of the endogenous gene associated with the bound DNA.

16. The method of claim 15, wherein the isolating comprises cross-linking the
20 artificial protein to the DNA, and immunoprecipitating the artificial protein.

17. The method of claim 15, further comprising identifying a homolog of the endogenous gene in a second type of cell, and regulating the expression of the homolog.

18. The method of claim 17, wherein the second type of cell is a prokaryotic cell.
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19. The method of claim 18, wherein the second type of cell is a bacterial cell.

20. A method comprising:
30 providing a plurality of prokaryotic cells, wherein each cell of the plurality comprises a nucleic acid encoding an artificial polypeptide, wherein the artificial polypeptide

comprises a zinc finger domain, and wherein the artificial polypeptide differs among the cells of the plurality;

identifying from the plurality a cell that has a trait that is altered relative to a reference cell.

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21. The method of claim 20, wherein the trait is tolerance to an organic solvent, and wherein the identifying comprises exposing cells of the plurality to the organic solvent and evaluating survival of the cells.

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22. The method of claim 20, wherein the trait is heat tolerance, and wherein the evaluating comprises exposing the cells to heat.

23. The method of claim 20, further comprising isolating the nucleic acid encoding the artificial polypeptide from the identified cell.

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24. The method of claim 23, further comprising sequencing the nucleic acid.

25. The method of claim 20, further comprising isolating the artificial polypeptide from the identified cell.

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26. The method of claim 20, further comprising isolating the nucleic acid encoding the artificial polypeptide from the identified cell, introducing the nucleic acid into a second plurality of cells, culturing the cells of the second plurality under conditions wherein the artificial polypeptide is produced, and identifying a cell of the second plurality having a trait that is altered relative to a reference cell.

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27. The method of claim 20, further comprising determining the sequence of the target DNA site of the artificial polypeptide.

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28. The method of claim 20, further comprising identifying an endogenous gene bound by the artificial polypeptide.

29. The method of claim 20, further comprising analyzing the expression of one or more genes of the cell.

30. The method of claim 28, further comprising modifying expression of the
5 endogenous gene in a second cell.

31. The method of claim 20, wherein the artificial polypeptide comprises at least three zinc finger domains.

10 32. The method of claim 31, wherein the zinc finger domains are yeast zinc finger domains, or variants thereof.

33. The method of claim 20, further comprising cultivating the identified cell to exploit the altered trait.

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34. A prokaryotic cell comprising:
a nucleic acid encoding an artificial polypeptide, wherein the artificial polypeptide comprises a zinc finger domain, and wherein the artificial polypeptide binds to a target DNA site in a gene and regulates expression of the gene under conditions in which the
20 nucleic acid is expressed.

35. The cell of claim 34, wherein the artificial polypeptide regulates expression of an endogenous gene.

25 36. The cell of claim 34, wherein the artificial polypeptide comprises at least three zinc finger domains.

37. The cell of claim 35, wherein the gene is a decarboxylase.

30 38. A cell selected by the method of claim 20.

39. A polypeptide comprising at least one zinc finger domain, wherein the DNA contacting residues of the zinc finger domain at positions -1, +2, +3, and +6 correspond to a motif selected from: RSHR, HSSR, ISNR, RDHT, QTHR, VSTR, QNTQ, and CSNR, and wherein the polypeptide regulates an endogenous prokaryotic gene.

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40. The polypeptide of claim 39, further comprising a second and third zinc finger domain, wherein the DNA contacting residues of the first, second, and third domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs RSHR, HSSR, and ISNR.

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41. The polypeptide of claim 39, further comprising a second and third zinc finger domain, wherein the DNA contacting residues of the first, second, and third domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs ISNR, RDHT, and QTHR.

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42. The polypeptide of claim 41, further comprising a fourth zinc finger domain, wherein the DNA contacting residues of the fourth domain at positions -1, +2, +3, and +6 of correspond to the motif VSTR.

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43. The polypeptide of claim 39, further comprising a second and third zinc finger domain, wherein the DNA contacting residues of the first, second, and third domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs QNTQ, CSNR, and ISNR.

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44. A polypeptide comprising at least one zinc finger domain, wherein the DNA contacting residues of the zinc finger domain at positions -1, +2, +3, and +6 correspond to a motif selected from: QSHV, VSNV, QSNK, RDHT, QTHR, QSSR, WSNR, VSNV, RSHR, DSAR, QTHQ, RSHR, QSNR, and CSNR, and wherein the polypeptide regulates an endogenous prokaryotic gene.

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45. The polypeptide of claim 44, further comprising a second, third, and fourth zinc finger domain, wherein the DNA contacting residues of the first, second, third, and

fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs QSHV, VSNV, QSNK, and QSNK.

46. The polypeptide of claim 44, further comprising a second, third, and fourth
5 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs RDHT, QSHV, QTHR, and QSSR.

47. The polypeptide of claim 44, further comprising a second, third, and fourth
10 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs WSNR, QSHV, VSNV, and QSHV.

48. The polypeptide of claim 44, further comprising a second, third, and fourth
15 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs QTHR, RSHR, QTHR, and QTHR.

49. The polypeptide of claim 44, further comprising a second, third, and fourth
20 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs DSAR, RDHT, QSHV, and QTHR.

50. The polypeptide of claim 44, further comprising a second, third, and fourth
25 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs QTHQ, RSHR, QTHR, and QTHR.

51. The polypeptide of claim 44, further comprising a second, third, and fourth
30 zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs QSHV, VSNV, QSNR, and CSNR.

52. The polypeptide of claim 44, further comprising a second, third, and fourth zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs VSNV, QTHR, QSSR, and RDHT.

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53. The polypeptide of claim 44, further comprising a second, third, and fourth zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs RDHT, QSHV, QTHR, and QSNR.

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54. The polypeptide of claim 44, further comprising a second, third, and fourth zinc finger domain, wherein the DNA contacting residues of the first, second, third, and fourth domains at positions -1, +2, +3, and +6 of each domain respectively correspond to the motifs DSAR, RDHT, QSNK, and QTHR.

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55. A nucleic acid encoding the polypeptide of claim 39.

56. A bacterial expression vector comprising a nucleic acid encoding the polypeptide of claim 39.